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ABSTRACT

Background: Schwannomatosis is a recently recognized third major type of neurofibromatosis. Our preliminary studies of the *N* gene in tumors from schwannomatosis patients reveal a pattern of tumor suppressor gene inactivation not previously reported in ar other human disease. **Objective/Hypothesis:** The objective of this project is to clone the locus responsible for familial schwannomatosis. We are exploring two competing hypotheses which address both the non random distribution of LOH observe schwannomatosis tumors and the high rate of somatic *NF2* mutation seen along the *cis* allele.

Specific Aims:

1. To identify and clinically characterize schwannomatosis patients, and maintain a resource of tumor and blood specimens.
2. To further refine the candidate region on chromosome 22 using linkage and loss of heterozygosity analyses.
3. To determine the molecular mechanism of tumor formation in these patients using complementary molecular and cytogen approaches.

Study Design: Schwannomatosis patients and affected relatives will be identified. Blood and tumor specimens will be obtained f linkage, LOH, FISH and mutational analysis of coding and non coding candidate regions. **Relevance:** This study will elucidate unique pathogenesis of schwannomatosis and provide a means for definitive diagnosis using molecular technology.

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Introduction

Neurofibromatosis (NF) encompasses a diverse group of genetic conditions whose common element is tumors of the nerve sheath. Schwannomatosis is a recently recognized third major type of NF, which results in multiple schwannomas without vestibular tumors diagnostic of NF2. Recent epidemiological studies have shown that schwannomatosis is as common as NF2. Our preliminary studies of the *NF2* gene in tumors from schwannomatosis patients revealed a pattern of tumor suppressor gene inactivation not previously been reported in any other human disease. The objective of this project is to clone the locus responsible for familial schwannomatosis, *sch*. We are exploring two competing hypotheses which address both the non random distribution of LOH observed in schwannomatosis tumors and the high rate of somatic *NF2* mutation seen along the *cis* allele. The first hypothesis is that *sch* is a second tumor suppressor gene which lies near to *NF2* on chromosome 22. In this model, schwannoma formation is dependent on four "hits" (two in the *sch* tumor suppressor, and two in the linked *NF2* tumor suppressor). FISH results have suggested a second hypothesis in which a structural element facilitates loss of the *trans* chromosome by increasing the rate of mitotic recombination. This is an especially attractive hypothesis since rates of mitotic recombination are both highly variable and genetically determined in humans.

Body

This section is organized around the approved statement of work.

Task 1. To develop a resource of study subjects and related biological materials (months 1 through 30):

a. Identify schwannomatosis probands to include two potential familial clusterings and five sporadic patients in each 8 month period (months 1 thorough 24).

Two new kindreds have been identified in the reporting period (coded as Family # E and Family # MGH-M). Two new sporadic patients were identified and enrolled.

Because of anecdotal reports of increasing severity of disease in subsequent generations, a formal analysis of the possibility of anticipation in schwannomatosis was performed by record review. We identified 24 affected parent-affected child from 11 families in which the parent was a non founder (to exclude the possibility of mosaicism causing anticipation). The average parent decade of onset was 3.65 while the average child decade of onset was 1.85. The average parent age of first surgery was 38.29 years while the average child age of first surgery was 25 years. Because increased awareness of disease symptoms and improved diagnostic methodology might impact on these numbers, we are currently generating a control group of individuals with another tumor suppressor gene syndrome of adult onset and known underlying mutation to determine the significance of these findings.

b. Obtain specimens from confirmed probands and affected relatives (months 6 through 30).

A total of 23 blood specimens were collected from newly identified familial clusterings, newly identified sporadic cases and expansion of previously identified familial clusterings. 13 tumor specimens from clinically indicated surgery were obtained. One patient in family 9 died and the family consented to autopsy collection of affected and unaffected tissues for study. This was performed on site by a study technician at the treating hospital. We have reduced our collection of paraffin embedded tumor material because of our success in recruiting fresh tumor material.

c. Perform NF2 mutational analysis on selected tumor specimens from confirmed probands (months 12 through 30).

We are nearing completion of mutational analysis on an additional 20 tumor specimens. This will be an especially valuable data set to extend our previous observations because all specimens are from familial patients and all were received in the laboratory flash frozen (which will allow much greater capability for future study over our previous work that was completed on archived paraffin specimens). In this group of tumors total of 10 mutations have been confirmed in exons 2, 3, 7, 8, 9, 10, 11 and 13 (two mutations were detected in exons 2 and 8). This mutation distribution confirms our previous impression that alterations of the conserved 5' ERM domain are much more common in schwannomatosis tumors then in non schwannomatosis tumors (figure 1). We have located the first non truncating mutation in a schwannomatosis patient to our knowledge (exon 2, six basepair deletion in a tumor from family V) and have tentatively identified an unprecedented "second hit" in this tumor. We continue to observe a lack of mutation in paired unaffected tissues (blood) although a rare putative polymorphism in intron 12 has been detected in family E tracking with the affected state.

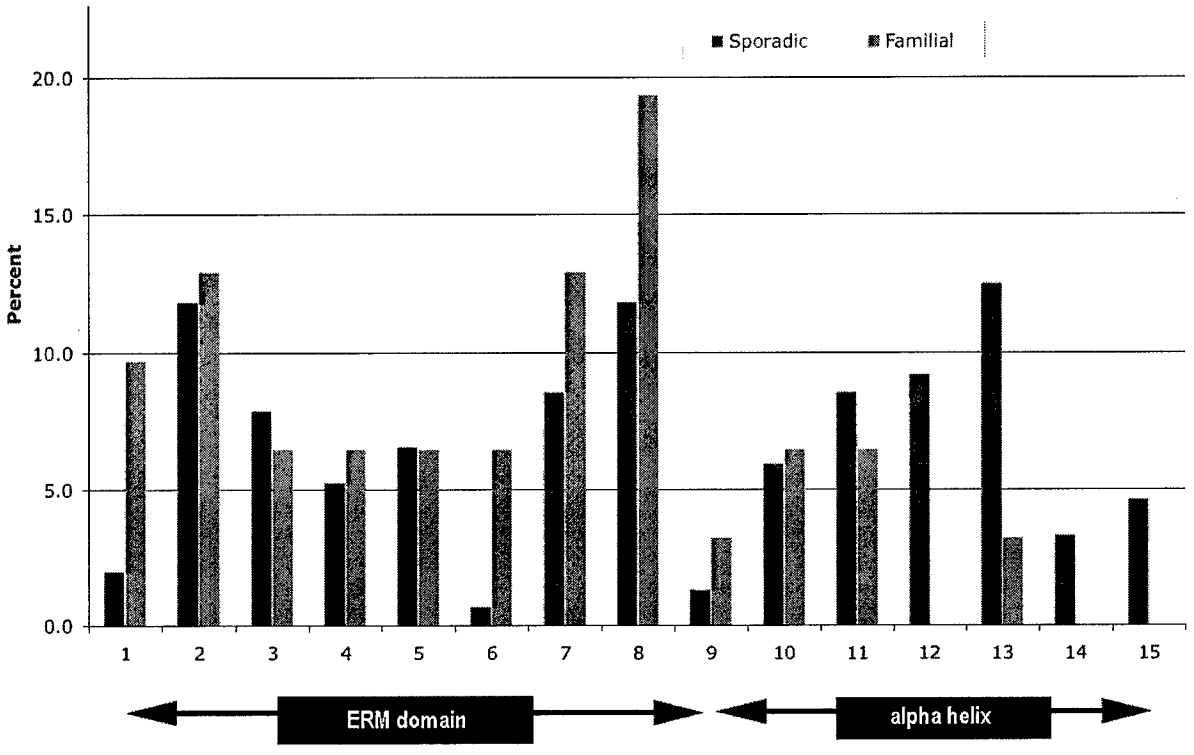

Figure 1. Distribution of mutations within the *NF2* gene. The percent of mutations involving each *NF2* exon in 152 sporadically occurring tumors cataloged in a separate project in the PI's laboratory (http://neurosurgery.mgh.harvard.edu/NFclinic/NFresearch.htm) is compared to that of 30 tumors from familial schwannomatosis patients studied in this project. Although sporadic tumors bear mutations throughout the *NF2* gene, those from familial schwannomatosis patients are clustered in the 5' ERM (ezrin-radixin-moisin like) region. The type of mutation (frameshifting, nonsense, splice site and non truncating) did not significantly differ between the two groups of tumors.

Task 2. To refine the candidate region using LOH and linkage analysis (months 12 through 36):

a. Perform LOH analysis on tumor specimens from confirmed probands, including 10 paraffin blocked specimens and 6 frozen tumors per 12 month time period (months 12 through 36)
LOH analysis is nearing completion on the set of 20 tumor specimens studied in task 1.c. above with loss seen in 18 of 20 tumors (90%) pending confirmatory study.

We have begun to use LOH analysis to narrow a candidate region by assessing for regions that are retained (and thus exclude a tumor suppressor gene) adjacent to areas that show loss. Twenty frozen tumor specimens from familial schwannomatosis patients were studied at the telomeric candidate region markers D22S1174 and D22S421. 16 of 20 tumors were lost at both markers and 4 of 20 were retained with no tumor splitting the region. However, a single tumor was found to be retained at a more centromeric marker (D22S264) while being lost at D22S1174 suggesting

exclusion of the more proximal portion of our candidate region. We are currently working towards refining the pattern of loss and retention in this tumor.

b. Perform linkage analysis on newly identified families, including 2 to 3 affected kindreds in each 12 month time period (months 12 through 36).

Excellent progress has been made regarding this task, leading to the current candidate region map shown in figure 1. We are concentrating intensively on the area from D22S264 to D22S1148 to further refine this map and are developing a number of polymorphic markers to do so (table 2).

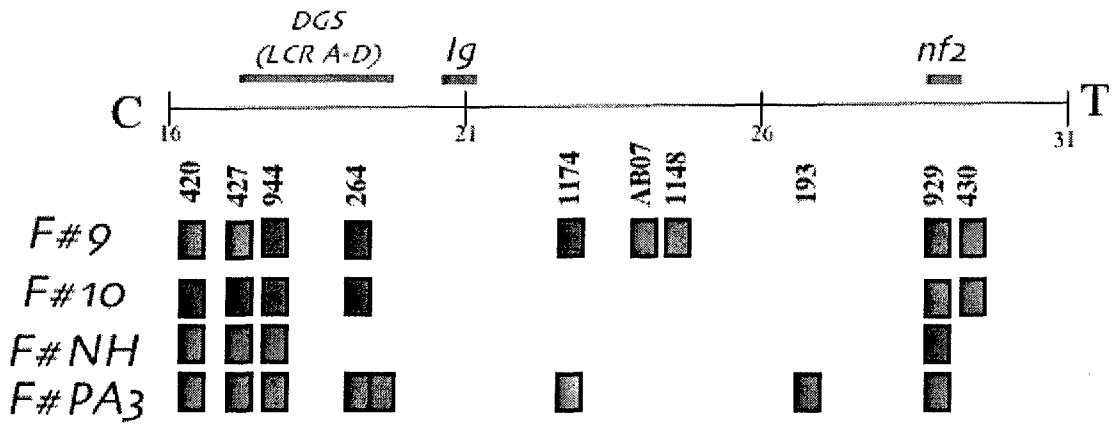

Figure 2. The best evidence candidate region. A 15 megabase region from the proximal portion of the long arm of chromosome 22 is shown to scale from the centromere (C) to telomere (T). The baseline is marked from 16 megabases to 31 megabases as denoted in build 35.1 of the human genome project accessed in the NCBI MapViewer function (http://www.ncbi.nlm.nih.gov/mapview). Anonymous microsattelite polymorphisms are shown below the baseline, and are boxed in green when shared by all affected family members and red when not shared. The candidate region comprises 5.4 basepairs from the markers D22S264/PCQAP not shared in affected family PA3 members to the markers AB07/D22S1148 which is not shared in family 9 affected members. This region contains two of the four DiGeorge region (DGS) low copy repeats (LCR C and D), the immunoglobulin super locus and approximately 60 known genes.

Assay Name	Chr 22 Location	# Alleles	% Heterozygous	Gene
AB-04	19,446,679	5	70.6%	*PIK4CA* Intron
AB-02	22,288,637	2	52.6%	*CR456370* Exon 4
AB-05	22,362,030	3	73.7%	*Rgr* Intron
AB-09	22,763,821	8	72.2%	*Cabin1* Intron
AB-08	22,813,196	5	52.6%	*Cabin1* Intron
AB-03	22,834,425	2	22.2%	*Cabin1* Intron
AB-07	24,095,992	8	82.4%	NONE

Table 1. Highly polymorphic microsatellites within the candidate schwannomatosis region. Assays have been developed surrounding areas of di-, tri- and tetra- nucleotide repeats detected by the Genome Browser Gateway of the UCSC Genome Bioinformatics service (http://genome.ucsc.edu) within the best evidence candidate region shown in figure 2. Allele number and percent of heterozygous individuals is given for those areas found to be polymorphic in a cohort of 13 non founding schwannomatosis patients and 6 normal controls. Interestingly, six of the seven simple repeats found to be polymorphic were located within genes.

Task 3. To determine the molecular mechanism leading to schwannomatosis (months 1 through 48):

c. Analysis of LOH patterns in 20 sporadic and 20 NF2 related non vestibular tumors and correlation with FISH analysis (months 12 to 24)

FISH analysis was completed in the laboratory of Dr. Arie Perry on 12 sporadic non vestibular tumors (from genetically normal individuals) and 20 NF2 related non vestibular schwannomas using the 12 probes developed and described in our previous report. Reproducible FISH data was achieved in 31 of 32 cases. 29 of 31 cases showed either two signals at all markers tested (17 cases) or one signal at all markers tested (12 cases). Two cases showed a mixture of retained and deleted signals. LOH patterns were then compared to the FISH results and unexpectedly in 5 of 8 tumors with two signals, LOH consistent with mitotic recombination was seen. These results conflict with older literature reports that monosomy is the primary mechanism behind LOH in schwannomas (Wolff et al., 1992), but are remarkably consistent with a more recent report finding isodisomy as the mechanism of LOH in 37% of vestibular schwannomas (Warren et al., 2003). To determine if these conflicting observations stem from differences in technique or true

differences between vestibular and non vestibular schwannomas, we are now determining the molecular mechanism of LOH in a group of vestibular tumors of both sporadic and NF2 origin.

Key Research Accomplishments

- Identification of two new schwannomatosis kindreds with significant sample collection
- Tentative identification of genetic anticipation in schwannomatosis families
- Identification of altered exonic distribution of mutation in familial schwannomatosis tumors.
- Further refinement of schwannomatosis candidate region and development of seven highly polymorphic satellite markers within the region.
- Identification of isodisomy as a mechanism of LOH in non vestibular tumors in general

Reportable Outcomes

Presentations:

Invited presentations by the PI concerning this research were made to the Pfizer research group, Groton campus (7/24/04), the Henry Ford Hospital Neuro Oncology research group (3/16/05) and the Children's Tumor Foundation (6/6/05).

Poster presentation: Webster MT, Larson KE, Maccollin M. Unequal expression assay as a rapid screen for candidate tumor suppressor genes, 54th Annual meeting of the American Society of Human Genetics, October, 2004 (abstract 1593).

Publications:

MacCollin M, Chiocca EA, Evans DG, Friedman JM, Horvitz R, Jaramillo D, Lev M, Mautner VF, Niimura M, Plotkin SR, Sang CN, Stemmer-Rachamimov A, Roach ES. Diagnostic criteria for schwannomatosis. Neurology. 2005 Jun 14;64(11):1838-45.

Funding applied for:

The PI supported the submission of a proposal on "Identification of molecular and histological markers for schwannomatosis associated schwannomas" by Dr. Anat Stemmer-Rachamimov to the NFRP (proposal log number NF050113) in February, 2005. The PI supported the submission of proposals titled "Molecular Pathology and Expression Profiling of Schwannomatosis Related Tumors" (PI: Dr. Anat Stemmer Rachamimov) and "A Phase II Trial of a Tumor Necrosis Factor (TNF-alpha) Antagonist in Schwannomatosis" (PI: Dr. Christine M Sang) to the Children's Tumor Foundation (also known as the National Neurofibromatosis Foundation) in April, 2005 Dr. Stemmer-Rachamimov's proposal was funded by the CTF in June, 2005.

Research opportunities:

The PI sponsored Summer Research Trainee Program student Andria Balogh, "Clinical and molecular investigation of anticipation in schwannomatosis" June-August, 2003.

Conclusions

Schwannomatosis is a third major form of NF, which recent epidemiological studies have shown is as common as NF2. However, clinical recognition and molecular characterization have lagged far behind other forms of NF. The clarification of molecular alterations in schwannomatosis will likely have broad implications for other tumor suppressor gene syndromes. We have identified significant resources over the past year to aid in the search for the fundamental alteration in schwannomatosis.

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Wolff RK, Frazer KA, Jackler RK, Lanser MJ, Pitts LH, Cox DR. Analysis of chromosome 22 deletions in neurofibromatosis type 2-related tumors. Am J Hum Genet. 1992;51:478-85.